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Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. C.R.116
Patent No. 7,803,383

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Richard Joseph Fagan, Andrew Robert Davids, Christopher Benjamin Phelps, Christine Power, Ursula Boschert, Yolande Chvatchko
Issued	:	September 28, 2010
Patent No.	:	7,803,383
Conf. No.	:	1499
For	:	Method of Treatment Comprising Administration of a Cytokine Antagonist Molecule

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 3, line 10:	Page 3, line 30:
"R W Sernin"	--R W Semin--

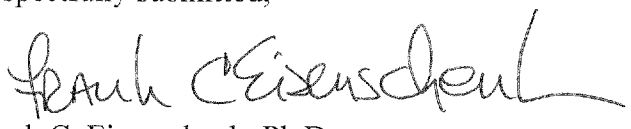
Column 3, line 39:	Page 4, line 18:
"57-68, Matsuno"	--57-68; Matsuno--
Column 6, line 54:	Page 8, line 15:
"IL-Ira"	--IL-1ra--
Column 7, line 34:	Page 9, line 15:
"cytoldnes"	--cytokines--
Column 9, line 52:	Page 12, line 26:
"1NSP052"	--INSP052--
Column 15, line 45:	Page 21, line 21:
"cytoline"	--cytokine--
Column 16, line 40:	Page 23, line 2:
"fimgal infection"	--fungal infection--
Column 25, line 28:	Page 35, line 29:
"(1988);."	--(1988);--
Column 35, line 7:	Page 49, line 31:
"Immunex"	--(Immunex--
Column 41, line 36:	Page 59, line 15:
"bacterial. toxoid"	--bacterial toxoid--
Column 43, line 27:	Page 62, line 6:
"Proc. Nati."	--Proc. Natl.--
Column 44, line 66:	Page 64, line 17:
"inyention"	--invention--

Column 45, line 29:	Page 65, line 7:
"bums"	--burns--
Column 46, line 20:	Page 66, line 9:
"HPBMC"	--hPBMC--
Column 46, line 28:	Page 66, line 19:
"protein in The IC50"	--protein in μg/ml). The IC50--
Column 48, line 33:	Page 69, line 25:
"IgGI"	--IgG1--
Column 53, line 19:	Page 75, line 31:
"IFN-garnma"	--IFN-gamma--
Column 53, line 67:	Page 77, line 2:
"25 μof"	--25 μl of--
Column 54, line 46:	Page 78, line 2:
"CDNA"	--cDNA--
Column 54, line 64:	Page 78, line 15:
"already show"	--already shown--.

A true and correct copy of pages 3, 4, 8, 9, 12, 21, 23, 35, 49, 59, 62, 64-66, 69, 75, 77, and 78 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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Attachments: Copy of pages 3, 4, 8, 9, 12, 21, 23, 35, 49, 59, 62, 64-66, 69, 75, 77, and 78 of the specification

processes. Alteration of their activity is a means to alter the disease phenotype and as such identification of novel immunoglobulin domain-containing cell surface recognition molecules is highly relevant as they may play a role in many diseases, particularly inflammatory disease, oncology, and cardiovascular disease. Immunoglobulin domain-containing cell surface recognition molecules are involved in a range of biological processes, including: embryogenesis (Martin-Bermudo, M.D. *et al,* Development. 2000 127(12):2607-15; Chen, L.M., *et al.*, J Neurosci. 2000 20(10):3776-84; Zweegman, S., *et al,* Exp Hematol. 2000 28(4):401-10; Darribere, T., *et al.,* Biol Cell. 2000 92(1):5-25), maintenance of tissue integrity (Eckes, B., *et al.,* J Cell Sci. 2000 113(Pt 13):2455-2462; Buckwalter, J.A., *et al.,* Instr Course Lect. 2000 49:481-9; Frenette, P.S., *et al.,*. J Exp Med. 2000 191(8):1413-22; Delmas, V., *et al,* Dev Biol. 1999 216(2):491-506; Humphries, M.J., *et al.,* Trends Pharmacol Sci. 2000 21(1):29-32; Miosge, N., *et al,* Lab Invest. 1999 79(12):1591-9; Nagaoka T, *et al.* Am J Pathol 2000 Jul 157:1 237-47; Nwariaku FE, *et al.* J Trauma 1995 39(2): 285-8; Zhu X, *et al.* Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi 1999 15(1): 53-5), leukocyte extravasation/inflammation (Lim, L.H., *et al.* Am J Respir Cell Mol Biol. 2000 22(6):693-701; Johnston, B., *et al.,* Microcirculation. 2000 7(2):109-18; Mertens, A.V., *et al.,* Clin Exp Allergy. 1993 23(10):868-73; Chcialowski, A., *et al.,* Pol Merkuriusz Lek. 2000 7(43):13-7; Rojas, A.I., *et al,* Crit Rev Oral Biol Med. 1999 10(3):337-58; Marinova-Mutafchieva, L., *et al.,* Arthritis Rheum. 2000 43(3):638-44; Vijayan, K.V., *et al,* J Clin Invest. 2000 105(6):793-802; Currie, A.J., *et al,*. J Immunol. 2000 164(7):3878-86; Rowin, M.E., *et al.*, Inflammation. 2000 24(2):157-73; Johnston, B., *et al.,* J Immunol. 2000 164(6):3337-44; Gerst, J.L., *et al.,* J Neurosci Res. 2000 59(5):680-4; Kagawa, T.F., *et al.,* Proc Natl Acad Sci U S A. 2000 97(5):2235-40; Hillan, K.J., *et al.,* Liver. 1999 19(6):509-18; Panes, J., 1999 22(10):514-24; Arao, T., *et al.,* J Clin Endocrinol Metab. 2000 85(1):382-9; Souza, H.S., *et al.,* Gut. 1999 45(6):856-63; Grunstein, M.M., *et al.,* Am J Physiol Lung Cell Mol Physiol. 2000 278(6):L1154-63; Mertens, A.V., *et al.,* Clin Exp Allergy. 1993 23(10):868-73; Berends, C., *et al.,* Clin Exp Allergy. 1993 23(11):926-33; Fernvik, E., *et al.,* Inflammation. 2000 24(1):73-87; Bocchino, V., *et al.,* J Allergy Clin Immunol. 2000 105(1 Pt 1):65-70; Jones SC, *et al*, Gut 1995 36(5):724-30; Liu CM, *et al*, Ann Allergy Asthma Immunol 1998 81(2):176-80; McMurray RW Semin Arthritis Rheum 1996 25(4):215-33; Takahashi H, *et al* Eur J Immunol 1992 22(11): 2879-85; Carlos T, *et al* J Heart Lung Transplant 1992 11(6): 1103-8; Fabrega E, *et al*, Transplantation 2000 69(4): 569-73; Zohrens G, *et al*, Hepatology 1993 18(4): 798-802; Montefort S, *et al.* Am J Respir Crit Care

Med 1994 149(5): 1149-52), oncogenesis (Orr, F.W., *et al.*, Cancer. 2000 88(S12):2912-2918; Zeller, W., *et al.*, J Hematother Stem Cell Res. 1999 8(5):539-46; Okada, T., *et al.*, Clin Exp Metastasis. 1999 17(7):623-9; Mateo, V., *et al.*, Nat Med. 1999 5(11):1277-84; Yamaguchi, K., *et al.*, J Exp Clin Cancer Res. 2000 19(1):113-20; Maeshima, Y., *et al.*, J Biol Chem. 2000 275(28):21340-8; Van Waes, C., *et al.*, Int J Oncol. 2000 16(6):1189-95; Damiano, J.S., *et al.*, Leuk Lymphoma. 2000 38(1-2):71-81; Seftor, R.E., *et al.*, Cancer Metastasis Rev. 1999 18(3):359-75; Shaw, L.M., J Mammary Gland Biol Neoplasia. 1999 4(4):367-76; Weyant, M.J., *et al.*, Clin Cancer Res. 2000 6(3):949-56), angiogenesis (Koch AE, *et al* Nature 1995 376 (6540): 517-9; Wagener C & Ergun S. Exp Cell Res 2000 261(1): 19-24; Ergun S, *et al.* Mol Cell 2000 5(2): 311-20), bone resorption (Hartman GD, & Duggan ME. Expert Opin Investig Drugs 2000 9(6): 1281-91; Tanaka Y, *et al.* J Bone Miner Res 1995 10(10): 1462-9; Lark MW, *et al.* J Pharmacol Exp Ther 1999 291(2): 612-7; Raynal C, *et al.* Endocrinology 1996 137(6):2347-54; Ilvesaro JM, *et al.* Exp Cell Res 1998 242(1): 75-83), neurological dysfunction (Ossege LM, *et al.* Int Immunopharmacol 2001 1:1085-100; Bitsch A, *et al*, Stroke 1998 29:2129-35; Iadecola C & Alexander M. Curr Opin Neurol 2001 14:89-94; Becker K, *et al* Stroke 2001 32(1): 206-11; Relton JK, *et al* Stroke 2001 32(1): 199-205; Hamada Y, *et al* J Neurochem 1996 66:1525-31), thrombogenesis (Wang, Y.G., *et al.*, J Physiol (Lond). 2000 526(Pt 1):57-68; Matsuno, H., *et al.*, Nippon Yakurigaku Zasshi. 2000 115(3):143-50; Eliceiri, B.P., *et al.*, Cancer J Sci Am. 2000 6(Suppl 3):S245-9; von Beckerath, N., *et al.*, Blood. 2000 95(11):3297-301; Topol, E.J., *et al.*, Am Heart J. 2000 139(6):927-33; Kroll, H., *et al.*, Thromb Haemost. 2000 83(3):392-6), and invasion/adherence of bacterial pathogens to the host cell (Dersch P, *et al.* EMBO J 1999 18(5): 1199-1213).

The detailed characterisation of the structure and function of several immunoglobulin-domain containing cell surface recognition molecule families has led to active programs by a number of pharmaceutical companies to develop modulators for use in the treatment of diseases involving inflammation, oncology, neurology, immunology and cardiovascular function. Immunoglobulin domain containing cell surface recognition molecules are involved in virtually every aspect of biology from embryogenesis to apoptosis. They are essential to the structural integrity and homeostatic functioning of most tissues. It is therefore not surprising that defects in immunoglobulin domain containing cell surface recognition molecules cause disease and that many diseases involve modulation of immunoglobulin

cytokine.

Preferably, the cytokine antagonists of the invention have immunomodulatory activity. By "immunomodulatory activity" is meant any activity detected *in vitro* or *in vivo* that affects the immune response. Examples of immunomodulatory activities includes immunosuppressive activities, anti-inflammatory activities, pro-apoptotic activities, anti-apoptotic activities and anti-tumoral activities.

Cytokines which may be inhibited by the cytokine antagonists of the invention include: TGF-alpha; EGF; members of the four alpha-helical bundle family of cytokine (such as IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-13, G-CSF, GM-CSF, CNTF, OSM, EPO, IL-10, IFN-alpha, IFN-beta, IFN-gamma and M-CSF); members of the cysteine knot family of cytokines (such as NGF, TGF-beta, PDGF and VEGF); chemokines (such as IL-8, MIP-1-alpha, MIP-1-beta, MIP-2, PF-4, PBP, I-309/TCA-3, MCP-1, MCP-2, MCP3 and IP-10), members of the TNF family of cytokines (such as TNF-alpha, TNF-beta and LT-beta); and members of the beta-trefoil family of cytokines including FGF-alpha, FGF-beta IL-1-alpha, IL-1-beta and IL-1ra). Preferred cytokine antagonists of the invention inhibit the activity of TNF-alpha, IL-4 and/or IL-2.

Cytokines form an heterogeneous family of secreted polypeptides, of relatively low molecular weight, acting as regulators of cellular reactions to various kind of stimuli, in particular those related to inflammatory and immune reactions but also those related to cell proliferation, repair, cell-cell interactions, and differentiation.

Cytokines are mainly represented by non-enzymatic glycoproteins that have been historically classified into several groups based on criteria such as sequence homology, structural elements, expressing cells, and/or binding activity. Examples of such families of sequences are Interleukins, Lymphokines, Monokines, Inteferons, but many other sequences have been characterized as having cytokine activities in the recent years (such as some growth factors or chemokines) making difficult to provide an exhaustive classification (Haddad JJ, 2002, Biochem Biophys Res Commun, 297:700-13).

Cytokines are mostly produced by monocytes, macrophages and lymphocytes, but also by other cell types (such as leukocytes, osteoblasts, smooth muscle cells, epithelial cells, neuronal cells, endothelial cells, or fibroblasts), but they are not normally produced in a constitutive manner. Cytokines are generally secreted by immune cells in response to an

offending agent (e.g. bacteria, virus, or other pathogens) that can stimulate their *de novo* expression and secretion from cells with the consequence of altering either their own functions (autocrine / intracrine effects) or those of adjacent cells and the local microenvironment (paracrine / juxtacrine effects).

Cytokines act on the target cell via receptors that trigger various cell signaling pathways. (Ishihara K and Hirano T, 2002, Biochim Biophys Acta, 1592: 281-296). Typically, the actions of cytokines are often pleiotropic (one cytokine may elicit several physiological effects), redundant (different cytokines may be responsible for a similar physiological effect), and may affect diverse and overlapping target cell populations.

The observed physiological effects of cytokines are due to fact that, in association to an inflammatory response leading to the mobilization and aggregation of cells, cytokines can induce (or suppress) the production of many proteins in a coordinate manner, including that of other cytokines and/or of cytokine receptors. Thus, many physiological responses (both under physiological and pathological conditions) are the result of inter-connected, redundant network of synergistic or antagonistic interactions amongst cytokines.

The cloning and biological analyses of cytokines and of their receptors have led to a general understanding of molecular basis behind their pleiotropism and redundancy. This property can be often ascribed to the composition of cytokine receptor complexes that include a signal-transducing receptor subunit that is used by all members of a cytokine family and a binding subunit that is specific for each cytokine. Thus, the cytokine-induced signalling cascade represents a mechanism having a final outcome for a particular cell or tissue that is determined by a number of different messages received concurrently at the cell surface.

The details of the functioning of the cytokine network are not yet fully understood, but it is clear that cytokines are important mediators and are implicated in the pathogenesis of numerous diseases, directly or indirectly related to both innate and adaptive immune responses. In particular, main patho-physiological effects of cytokines result from their excessive or inappropriately localized production, inducing an inflammatory state negatively affecting the state of a patient. Therefore, it is a generally acknowledged that any mechanism effectively inhibiting one or more cytokines, in particular proinflammatory cytokines, may have a positive effect in the treatment of human diseases by controlling the adverse cascade of cellular events associated to inappropriate or prolonged production of

based molecules.

The polypeptide having the sequence recited in SEQ ID NO:2 is referred to hereafter as "the INSP052 exon 1 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:4 is referred to hereafter as "the INSP052 exon 2 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:6 is referred to hereafter as "the INSP052 exon 3 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:8 is referred to hereafter as "the INSP052 exon 4 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:10 is referred to hereafter as "the INSP052 exon 5 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:12 is referred to hereafter as "the INSP052 exon 6 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:14 is referred to hereafter as "the INSP052 exon 7 polypeptide". Combining SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 produces the sequence recited in SEQ ID NO:16. The polypeptide having the sequence recited in SEQ ID NO:16 is referred to hereafter as the INSP052 polypeptide. The polypeptide having the sequence recited in SEQ ID NO:20 is the extracellular domain of INSP052. The polypeptide having the sequence recited in SEQ ID NO:22 is referred to hereafter as the extracellular domain of the mature INSP052 polypeptide. The polypeptide having the sequence recited in SEQ ID NO:24 is referred to hereafter as the mature INSP052 exon 2 polypeptide. The polypeptide having the sequence recited in SEQ ID NO:26 is referred to hereafter as the mature INSP052 polypeptide. The polypeptide having the sequence recited in SEQ ID NO:29 is referred to hereafter as the histidine-tagged, extracellular domain of mature INSP052. The polypeptide having the sequence recited in SEQ ID NO:30 is referred to hereafter as the Fc fusion of the extracellular domain of mature INSP052. The polypeptide having the sequence recited in SEQ ID NO:31 is referred to hereafter as the Ig domain containing fragment of INSP052 (INSP052Ig2).

The term "INSP052 exon polypeptides" as used herein includes polypeptides comprising or consisting of the polypeptide sequences set forth herein, including the INSP052 exon 1 polypeptide, the INSP052 exon 2 polypeptide, the INSP052 exon 3 polypeptide, the INSP052 exon 4 polypeptide, the INSP052 exon 5 polypeptide, the INSP052 exon 6 polypeptide, the INSP052 exon 7 polypeptide, the INSP052 polypeptide, the extracellular domain of INSP052, the extracellular domain of mature INSP052, the INSP052 mature exon 2 polypeptide, the mature INSP052 polypeptide, the histidine-tagged, extracellular

skilled person will understand that the term "ligand" encompasses any moiety that binds specifically to the polypeptide of the first aspect of the invention including, for example, antibodies and orphan receptors.

By "the activity of a polypeptide of the invention" and similar expressions, we refer to activity characteristic of immunoglobulin domain-containing cell surface recognition molecules. In particular, included within this definition is activity as a cytokine antagonist as defined above, particularly as an antagonist of cytokine expression and/or secretion, particularly with respect to TNF-alpha, IL-4 and IL-2. Preferably, the cytokine antagonists of the invention have immunomodulatory activity. An example of a utility of the polypeptides of the invention in this respect is in the treatment of T cell-mediated inflammation of the skin, such as in allergic contact dermatitis and psoriasis.

Methods for identification of ligands according to the sixth aspect of the invention are described in detail below. In particular, the invention provides a method for the identification of a compound that is a ligand that binds specifically to the polypeptides of SEQ ID NO: 20 or SEQ ID NO: 22, comprising contacting a polypeptide according to the first aspect of the invention with one or more compounds suspected of possessing binding affinity for said polypeptide and selecting a compound that binds specifically to said polypeptide. Ligands of the polypeptides of the invention identified by such methods may be used to identify further cytokine antagonists. For example, screening methods may be contacted to identify compounds that bind to such ligands, such as antibodies, which may also act as cytokine antagonists.

In a seventh aspect, the invention provides a compound that is effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

A compound of the seventh aspect of the invention may either increase (agonise) or decrease (antagonise) the level of expression of the gene or the activity of the polypeptide.

Importantly, the identification of the function of the INSP052 and INSP055 polypeptides allows for the design of screening methods capable of identifying compounds that are effective in the treatment and/or diagnosis of disease. Ligands and compounds according to the sixth and seventh aspects of the invention may be identified using such methods. These methods are included as aspects of the present invention.

disease, motor neurone disease, neuromuscular disease, HIV, AIDS, cytomegalovirus infection, fungal infection, ocular disorder, macular degeneration, glaucoma, diabetic retinopathy, ocular hypertension and other conditions in which immunoglobulin domain containing cell surface recognition molecules, particularly cytokine antagonists, are implicated.

It is particularly preferred that the moieties of the first, second, third, fourth, fifth and sixth aspects of the invention are used in the manufacture of a medicament for the treatment of inflammatory diseases, auto-immune diseases, skin disease, liver disease (including viral or acute liver disease) and liver failure (including alcoholic liver failure).

The moieties of the first, second, third, fourth, fifth and sixth aspects of the invention may be used in combination with other therapeutic agents to treat the diseases listed above. In particular, the polypeptides of the invention (including fusion proteins) which have cytokine antagonist activity may be used in combination with a further therapeutic agent.

The invention therefore provides the use of i) a moiety of the first, second, third, fourth, fifth and sixth aspects of the invention, in particular a polypeptide or a fusion protein comprising the extracellular domain of INSP052 and ii) a further therapeutic agent, in the manufacture of a medicament for treating diseases or conditions in which cytokines, preferably TNF, IL-2 and/or IL-4, are implicated. Preferably, the disease or condition is selected from inflammatory diseases, auto-immune diseases, skin disease, liver disease (including viral or acute liver disease) and liver failure (including alcoholic liver failure).

The further therapeutic agent may antagonise the same cytokine as the polypeptides of the invention or may antagonise another moiety in the physiological pathway causing the disease or alleviate symptoms caused by the disease.

Preferably, the therapeutic agent is a cytokine antagonist (preferably an antagonist of TNF, IL-2 and/or IL-4), or an anti-inflammatory agent.

Examples of cytokine antagonists which may be used as further therapeutic agents include TNF-alpha antagonists such as etanercept, infliximab TNF alpha converting enzyme inhibitor (TACE inhibitor), and leflunomide used in the treatment of rheumatoid arthritis; and IL-2 antagonists such as basilimab and daclizumab used in the prevention of graft rejection. The further therapeutic agent may also be an anti-inflammatory agent such as a

fold, 10^5-fold or 10^6-fold greater for a polypeptide of the invention than for known immunoglobluin domain-containing cell surface recognition molecules.

If polyclonal antibodies are desired, a selected mammal, such as a mouse, rabbit, goat or horse, may be immunised with a polypeptide of the first aspect of the invention. The polypeptide used to immunise the animal can be derived by recombinant DNA technology or can be synthesized chemically. If desired, the polypeptide can be conjugated to a carrier protein. Commonly used carriers to which the polypeptides may be chemically coupled include bovine serum albumin, thyroglobulin and keyhole limpet haemocyanin. The coupled polypeptide is then used to immunise the animal. Serum from the immunised animal is collected and treated according to known procedures, for example by immunoaffinity chromatography.

Monoclonal antibodies to the polypeptides of the first aspect of the invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies using hybridoma technology is well known (see, for example, Kohler, G. and Milstein, C., Nature 256: 495-497 (1975); Kozbor *et al.*, Immunology Today 4: 72 (1983); Cole *et al.*, 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985).

Panels of monoclonal antibodies produced against the polypeptides of the first aspect of the invention can be screened for various properties, i.e., for isotype, epitope, affinity, etc. Monoclonal antibodies are particularly useful in purification of the individual polypeptides against which they are directed. Alternatively, genes encoding the monoclonal antibodies of interest may be isolated from hybridomas, for instance by PCR techniques known in the art, and cloned and expressed in appropriate vectors.

Chimeric antibodies, in which non-human variable regions are joined or fused to human constant regions (see, for example, Liu *et al.*, Proc. Natl. Acad. Sci. USA, 84, 3439 (1987)), may also be of use.

The antibody may be modified to make it less immunogenic in an individual, for example by humanisation (see Jones *et al.*, Nature, 321, 522 (1986); Verhoeyen *et al.*, Science, 239, 1534 (1988); Kabat *et al.*, J. Immunol., 147, 1709 (1991); Queen *et al.*, Proc. Natl Acad. Sci. USA, 86, 10029 (1989); Gorman *et al.*, Proc. Natl Acad. Sci. USA, 88, 34181 (1991); and Hodgson *et al.*, Bio/Technology, 9, 421 (1991)). The term "humanised antibody", as

Alternatively, the sequences encoding the polypeptide of the invention may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesise RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH)).

Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes and fluorescent, chemiluminescent or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Nucleic acid molecules according to the present invention may also be used to create transgenic animals, particularly rodent animals. Such transgenic animals form a further aspect of the present invention. This may be done locally by modification of somatic cells, or by germ line therapy to incorporate heritable modifications. Such transgenic animals may be particularly useful in the generation of animal models for drug molecules effective as modulators of the polypeptides of the present invention.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography is particularly useful for purification. Well known techniques for refolding proteins may be employed to regenerate an active conformation when the polypeptide is denatured during isolation and or purification.

Specialised vector constructions may also be used to facilitate purification of proteins, as desired, by joining sequences encoding the polypeptides of the invention to a nucleotide sequence encoding a polypeptide domain that will facilitate purification of soluble proteins. Examples of such purification-facilitating domains include metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilised metals, protein A domains that allow purification on immobilised immunoglobulin, and the domain utilised in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor

Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics (1996), T Strachan and A P Read, BIOS Scientific Publishers Ltd).

Another approach is the administration of "naked DNA" in which the therapeutic gene is directly injected into the bloodstream or muscle tissue.

In situations in which the polypeptides or nucleic acid molecules of the invention are disease-causing agents, the invention provides that they can be used in vaccines to raise antibodies against the disease causing agent.

Vaccines according to the invention may either be prophylactic (ie. to prevent infection) or therapeutic (ie. to treat disease after infection). Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with pharmaceutically-acceptable carriers as described above, which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, H. pylori, and other pathogens.

Since polypeptides may be broken down in the stomach, vaccines comprising polypeptides are preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents.

The vaccine formulations of the invention may be presented in unit-dose or multi-dose containers. For example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Genetic delivery of antibodies that bind to polypeptides according to the invention may also be effected, for example, as described in International patent application WO98/55607.

differ by single nucleotides.

DNA sequence differences may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (for example, Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, Proc. Natl. Acad. Sci. USA (1985) 85: 4397-4401).

In addition to conventional gel electrophoresis and DNA sequencing, mutations such as microdeletions, aneuploidies, translocations, inversions, can also be detected by *in situ* analysis (see, for example, Keller *et al.*, DNA Probes, 2nd Ed., Stockton Press, New York, N.Y., USA (1993)), that is, DNA or RNA sequences in cells can be analysed for mutations without need for their isolation and/or immobilisation onto a membrane. Fluorescence *in situ* hybridization (FISH) is presently the most commonly applied method and numerous reviews of FISH have appeared (see, for example, Trachuck *et al.*, Science, 250, 559-562 (1990), and Trask *et al.*, Trends, Genet., 7, 149-154 (1991)).

In another embodiment of the invention, an array of oligonucleotide probes comprising a nucleic acid molecule according to the invention can be constructed to conduct efficient screening of genetic variants, mutations and polymorphisms. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee *et al.*, Science (1996), Vol 274, pp 610-613).

In one embodiment, the array is prepared and used according to the methods described in PCT application WO95/11995 (Chee *et al*); Lockhart, D. J. *et al.* (1996) Nat. Biotech. 14: 1675-1680); and Schena, M. *et al.* (1996) Proc. Natl. Acad. Sci. 93: 10614-10619). Oligonucleotide pairs may range from two to over one million. The oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support. In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application W095/25116 (Baldeschweiler *et al*). In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum

assays for the polypeptide include methods that utilise the antibody and a label to detect the polypeptide in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules known in the art may be used, several of which are described above.

Quantities of polypeptide expressed in subject, control and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease. Diagnostic assays may be used to distinguish between absence, presence, and excess expression of polypeptide and to monitor regulation of polypeptide levels during therapeutic intervention. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials or in monitoring the treatment of an individual patient.

A diagnostic kit of the present invention may comprise:

(a) a nucleic acid molecule of the present invention;

(b) a polypeptide of the present invention; or

(c) a ligand of the present invention.

In one aspect of the invention, a diagnostic kit may comprise a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to the invention; a second container containing primers useful for amplifying the nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease. The kit may further comprise a third container holding an agent for digesting unhybridised RNA.

In an alternative aspect of the invention, a diagnostic kit may comprise an array of nucleic acid molecules, at least one of which may be a nucleic acid molecule according to the invention.

To detect polypeptide according to the invention, a diagnostic kit may comprise one or more antibodies that bind to a polypeptide according to the invention; and a reagent useful for the detection of a binding reaction between the antibody and the polypeptide.

Such kits will be of use in diagnosing a disease or susceptibility to disease, including, but not limited to, diseases including, but not limited to, cell proliferative disorders,

autoimmune/inflammatory disorders, cardiovascular disorders, neurological and psychiatric disorders, developmental disorders, genetic disorders, metabolic disorders, infections and other pathological conditions. These diseases preferably include neoplasm, cancer, brain tumour, glioma, bone tumor, lung tumor, breast tumour, prostate tumour, colon tumour, hemangioma, myeloproliferative disorder, leukemia, hematological disease, neutropenia, thrombocytopenia, angiogenesis disorders, dermatological disease, ageing, wounds, burns, fibrosis, cardiovascular disease, restensosis, heart disease, peripheral vascular disease, coronary artery disease, oedema, thromboembolism, dysmenorrhea, endometriosis, pre-eclampsia, lung disease, COPD, asthma bone disease, renal disease, glomerulonephritis, skin disease, liver disease, Crohn's disease, gastritis, ulcerative colitis, ulcer, immune disorder, autoimmune disease, arthritis, rheumatoid arthritis, psoriasis, epidermolysis bullosa, systemic lupus erythematosus, ankylosing spondylitis, Lyme disease, multiple sclerosis, neurodegeneration, stroke, brain/spinal cord injury, Alzheimer's disease, Parkinson's disease, motor neurone disease, neuromuscular disease, HIV, AIDS, cytomegalovirus infection, fungal infection, ocular disorder, macular degeneration, glaucoma, diabetic retinopathy, ocular hypertension and other conditions in which immunoglobulin domain containing cell recognition molecules are implicated.

Various aspects and embodiments of the present invention will now be described in more detail by way of example, with particular reference to the INSP052 and INSP055 polypeptides.

It will be appreciated that modification of detail may be made without departing from the scope of the invention.

Brief description of the Figures

Figure 1: (A) Multiple alignment generated by CLUSTALW of the full-length INSP052 polypeptide sequence, the mature, isolated extracellular domain INSP052 (INSP052EC), and the closest related sequence identified in WO04/009834 (SEQIDNO434 and SEQIDNO880). (B) Multiple alignment generated by CLUSTALW comparing INSP052EC with the corresponding histidine tagged (INSP052EC-6His) and Fc fusion (INSP052EC-FC) versions of this sequence and with the Ig-domain-containing fragment of INSP052 (INSP052Ig2). The Fc sequence corresponds to amino acids 246-477 of the constant region

of human immunoglobulin lambda heavy chain IgG1; NCBI Acc. No. CAA75302. Underlined sequence denotes predicted signal peptide. Boxed sequence denotes predicted transmembrane domain. " * " indicates identical residues amongst the aligned sequences. " : " indicates homologous residues amongst the aligned sequences.

Figure 2: % of secreted TNF-alpha (TNF-a) by ConA-stimulated hPBMC mixed with serially diluted preparations of INSP052EC-6His (expressed in dilution of the protein preparation; see Example 4). The two curves (interpolating either crosses or circles) represent the results obtained with two different lots of the protein.

Figure 3: % secreted IL-4 (Interleukin 4) by ConA-stimulated hPBMC mixed with serially diluted preparations of INSP052EC-6His (expressed in dilution of the protein preparation; see Example 4). The two curves (interpolating either crosses or circles) represent the results obtained with two different lots of the protein.

Figure 4: % secreted IL-2 (Interleukin 2) by ConA-stimulated hPBMC mixed with serially diluted preparations of INSP052EC-6His (expressed in dilution of the protein preparation; see Example 4). The two curves (interpolating either crosses or circles) represent the results obtained with two different lots of the protein.

Figure 5: inhibition of IL-5 (Interleukin 5; A) and IL-2 (Interleukin 2; B) secretion ConA-stimulated hPBMC mixed with increasing amount of INSP052EC-6His (expressed in log of the concentration of the protein in μg/ml). The IC50 value is indicated by the line interpolating the curve and the X/Y axis.

Figure 6: inhibition of IL-5 (Interleukin 5; A) and IL-2 (Interleukin 2; B) secretion by ConA-stimulated purified CD4+ T cells mixed with increasing amount of INSP052EC-6His (expressed in μg/ml). The effect can be compared to the values of cytokine secretion in presence or absence of ConA (YES, NO), and in presence of both ConA and Dexamethasone (Dex; 0.1 mg/kg).

Figure 7: INSP052EC-electrotransferred animals (pDEST12.2 INSP052-6HIS) show a decrease of the blood levels of transaminases alanine aminotransferase (ALAT; A) and aspartate aminotransferase (ASAT; B) as compared to empty vector (pDEST12.2) control

The choice of one or more of these sequences to be fused to INSP052EC is functional to specific use and/or purification protocol of said protein as recombinant protein. For example, the activity of INSP052EC can be also test tested by means of a fusion protein including an albumin sequence or, as shown in the examples with INSP052EC-6HIS (SEQ ID NO: 29), a histidine tag sequence facilitating both detection and purification.

Alternatively, fusion proteins comprising INSP052EC can be obtained by linking this sequence to an immunoglobulin domain constant region, a protein domain known to improve the stability and the efficacy of recombinant proteins in the circulation. The resulting fusion protein can be expressed directly by mammalian cells (such as CHO or HEK293 cells) using the appropriate expression vectors so that the fusion protein is secreted in the culture medium.

Different strategies for generating fusion protein comprising an immunoglobulin fragment are disclosed in the literature (WO 91/08298; WO 96/08570; WO 93/22332; WO 04/085478; WO 02/66514). For example, the nucleic acid sequence encoding the mature INSP052EC can be cloned in an expression vector fused to a nucleic acid sequence encoding the original INSP052EC signal sequence (or any other appropriate signal sequence) at its 5' end, and the nucleic acid sequence encoding the constant region of human immunoglobulin lambda heavy chain IgG1 (NCBI Acc. No. CAA75302) at its 3' end (SEQ ID NO: 30). The resulting vector can be used to transform a CHO or HEK293 cell line and the clones stably expressing and secreting the recombinant fusion protein having INSP052EC at the N-terminus and the IgG1 sequence at the C-terminus can be selected. This clone then can be used for scaling up the production and for purifying the recombinant fusion protein from the culture medium. Alternatively, the position of the nucleic acid encoding the constant region of human immunoglobulin lambda heavy chain IgG1 and INSP052EC can be inversed, and the resulting protein can be expressed and secreted using still the original signal sequence of INSP052, or any other appropriate signal sequence.

Other protein sequences allowing the multimerization of INSP052EC are domains isolated from proteins such hCG (WO 97/30161), collagen X (WO 04/33486), C4BP (WO 04/20639), Erb proteins (WO 98/02540), or coiled coil peptides (WO 01/00814).

The additional sequence include in these fusion proteins may be eliminated at the end of its purification or in vivo, if needed, by means of an appropriate endo-/ exopeptidase. For

6.2: Background - Concanavalin A (ConA)-induced liver hepatitis

Toxic liver disease represents a worldwide health problem in humans for which pharmacological treatments have yet to be discovered. For instance active chronic hepatitis leading to liver cirrhosis is a disease state, in which liver parenchymal cells are progressively destroyed by activated T cells. ConA-induced liver toxicity is one of three experimental models of T-cell dependent apoptotic and necrotic liver injury described in mice. Gal N (D-Galactosamine) sensitized mice challenged with either activating anti-CD3 monoclonal AB or with superantigen SEB develop severe apoptotic and secondary necrotic liver injury (Kusters S, Gastroenterology. 1996 Aug;111(2):462-71). Injection of the T-cell mitogenic plant lectin ConA to non sensitized mice results also in hepatic apoptosis that preceeds necrosis. ConA induces the release of systemic TNF-alpha and IFN-gamma and various other cytokines. Both TNF-alpha and IFN-gamma are critical mediators of liver injury. Transaminase release 8 hours after the insult indicates severe liver destruction.

Several cell types have been shown to be involved in liver damage, CD4 T cells, macrophages and natural killer cells (Kaneko J Exp Med 2000, 191, 105-114). Anti-CD4 antibodies block activation of T cells and consequently liver damage (Tiegs et al. 1992, J Clin Invest 90, 196-203). Pre-treatment of mice with monoclonal antibodies against CD8 failed to protect, whereas deletion of macrophages prevented the induction of hepatitis.

The present study was undertaken to investigate the role of INSP052EC, a TNF-alpha / IL-4 cytokines antagonist protein containing IgG-like domains, in ConA-induced liver hepatitis. Several cytokines have been shown either to be critical in inducing or in conferring protection from ConA-induced liver damage. TNF-alpha for example is one of the first cytokines produced after ConA injection and anti-TNF-alpha antibodies confer protection against disease (Seino et al. 2001, Annals of surgery 234, 681-688). IFN-gamma appears also to be a critical mediator of liver injury, since anti-IFN-gamma antiserum significantly protect mice, as measured by decreased levels of transaminases in the blood of ConA-treated animals (see Kusters et al., above). In liver injury, increased production of IFN-gamma was observed in patients with autoimmune or viral hepatitis. In addition transgenic mice expressing IFN-gamma in the liver develop liver injury resembling chronic active hepatitis (Toyonaga et al. 1994, PNAS 91, 614-618). IFN-gamma may also be cytotoxic to hepatocytes, since *in vitro* IFN-gamma induces cell death in mouse

tibialis mucle with (20U in 50µl sterile NaCl 0.9% , Sigma Ref. H3631). After 10 minutes, 100 µg of plasmid (50 µg per leg in 25µl of sterile NaCl 0.9%) was injected in the same muscle. The DNA was prepared in the Buffer PBS-L-Glutamate (6mg/ml; L-Glutamate Sigma P4761) before intramuscular injection. For electrotransfer, the electric field was applied for each leg with the ElectroSquarePorator BTX ref ECM830 at 75 Volts during 20ms for each pulse, 10 pulses with an interval of 1 second in a unipolar way with 2 round electrodes (size 0.5mm diameter).

6.4.3: The ConA Model

6.4.3.1: ConA i.v. injection and blood sampling

8 weeks old Female Mice C57/Bl6 were purchased from IFFA CREDO. ConA (Sigma ref.C7275) was injected at 18mg/kg i.v., and blood samples were taken at 1.30 and 8 hours post-injection. At the time of sacrifice, blood was taken from the heart.

6.4.3.2: Detection of cytokines and transaminases in blood samples

IL2, IL5, IL4, TNF-alpha and IFN-gamma cytokine levels were measured using the TH1/TH2 CBA assay. TNF-alpha, IL-6, MCP1, IFN-alpha, IL-10 and IL-12 were detected using the Inflammation CBA assay. Transaminase (ALAT and ASAT) blood parameters were determined using the COBAS instrument (Hitachi).

6.4.3.3: Electrotransfer of the vectors expressing human INSP052EC-6His and hIL-6-SII

At day 0 electrotransfer of pDEST12.2.-INSP052EC, pDEST12.2-hIL-6 as well as and the empty vector control (electrotransfer protocol see above) was performed. At day 5 after electrotransfer, ConA (18 mg/kg) was injected i.v. and blood sampled at 2 time points (1.30, 8 hours).

6.4.3.4: INSP052 and IL6 protein pretreatment in the ConA model

CHO cell produced, recombinant hIL-6 or HEK293 cell produced, recombinant INSP052-6His was injected s.c. 30 minutes before ConA injection.

6.5: Results

We have shown previously (see Examples 4 and 5; Figures 2-6) that HEK 293 cell expressed INSP052EC-6His protein down-regulates TNF-alpha and IL-4 secretion (amongst other cytokines) in ConA stimulated hPBMC *in vitro* in a dose dependent way.

Since these two cytokines play a crucial role in T cell induced ConA induced liver hepatitis, we tested INSP052EC cDNA and protein in this model.

INSP052EC-6His protects from liver injury in a mouse model mimicking fulminant hepatitis after systemic delivery of the protein using electrotransfer. Figure 7 show that INSP052EC-6His -eletrotransferred animals show a decrease in transaminase levels as compared to empty vector control animals 8 hours after the ConA challenge. In addition both TNF-alpha and IL-6 cytokine levels are significantly reduced in these animals (Figure 8). Please note that the effect is similar, or even more important, to that obtained with the positive control vector pDEST12.2hIL-6-SII (Figures 7 and 8).

The protective effect of INSP052EC-6His was tested also by administering the purified recombinant protein before the injection of ConA. When s.c. injected, INSP052EC protein (1 mg/kg, or 0.3 mg/kg) decreased significantly ASAT and ALAT levels 8 hours after ConA injection (Figure 9).

6.6: Conclusion

Our experiments have already shown, that INSP052EC downregulates the secretion and/or expression of cytokines such as TNF-alpha, IL-4 and IL-2 *in vitro* in the ConA stimulated hPBMC assay. In addition, the delivery of INSP052EC cDNA in an *in vivo* model of fulminant hepatitis decreases TNF-alpha and m-IL-6 levels in serum and had a significant effect on the reduction of transaminases measured in serum, which was confirmed by s.c. INSP052EC protein injections.

The decrease in ASAT and ALAT levels might be due to both, decreased TNF-alpha and IL-4 levels. TNF-alpha and IL-4 are important cytokines involved in the liver damage after ConA injection. In this mouse model of liver hepatitis TNF-alpha is mainly produced by hepatic macrophages, the so-called Kupfer cells, whereas IL-4 is produced by liver (natural killer T) NKT cells. Anti TNF-alpha antibodies confer protection against disease (Seino et al. 2001, Annals of surgery 234, 681-688) and inhibition of IL-4 production by NKT cells was shown to be hepato-protective in T-cell mediated hepatitis in mouse (Ajuebor et al. 2003 J. Immunology 170, 5252-9).

INSP052EC might be useful in treating auto-immune, viral or acute liver diseases as well as alcoholic liver failures. It might be also effective in other inflammatory diseases.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,803,383

APPLICATION NO.: 10/579,113

DATED : September 28, 2010

INVENTORS : Richard Joseph Fagan, Andrew Robert Davids, Christopher Benjamin Phelps, Christine Power, Ursula Boschert, Yolande Chvatchko

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 3,
Line 10, "R W Sernin" should read --R W Semin--.
Line 39, "57-68, Matsuno" should read --57-68; Matsuno--.

Column 6,
Line 54, "IL-Ira" should read --IL-1ra--.

Column 7,
Line 34, "cytoldnes" should read --cytokines--.

Column 9,
Line 52, "1NSP052" should read --INSP052--.

Column 15,
Line 45, "cytoline" should read --cytokine--.

Column 16,
Line 40, "fimgal infection" should read --fungal infection--.

Column 25,
Line 28, "(1988);." should read --(1988);--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 35,
Line 7, "Immunex" should read –(Immunex--.

Column 41,
Line 36, "bacterial. toxoid" should read --bacterial toxoid--.

Column 43,
Line 27, "Proc. Nati." should read --Proc. Natl.--.

Column 44,
Line 66, "inyention" should read --invention--.

Column 45,
Line 29, "bums" should read --burns--.

Column 46,
Line 20, "HPBMC" should read --hPBMC--.
Line 28, "protein in The IC50" should read --protein in μg/ml). The IC50--.

Column 48,
Line 33, "IgGI" should read --IgG1--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 53,
Line 19, "IFN-garnma" should read --IFN-gamma--.
Line 67, "25 μof" should read --25 μl of--.

Column 54,
Line 46, "CDNA" should read --cDNA--.
Line 64, "already show" should read --already shown--.

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